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**14. ABSTRACT**
The receptor tyrosine kinase HER2/ ErbB2 is overexpressed in about 25% of all breast cancers, and presents an attractive therapeutic target. However, drug resistance is a significant clinical problem with current ErbB2-targeted therapies. The development of novel therapeutic strategies demands knowledge of ErbB2 receptor cross-talk with other signaling pathways. Recent reports have shown that Protein Tyrosine Phosphatase 1B (PTP1B) plays a positive role in ErbB2-induced breast cancer in vitroand in vivo. This research aims to understand the role of PTP1B-regulated pathways in ErbB2-mediated progression of breast cancer. The study uses quantitative proteomics to identify pro-oncogenic PTP1B substrates in ErbB2-transformed human mammary epithelial cells. Two cell populations—one with normal PTP1B expression (ErbB2/PTP1B+) and the other with reduced PTP1B expression (ErbB2/PTP1B-) were subjected to SILAC (Stable Isotope Labeling by Amino Acids in Cell Culture) coupled with quantitative phospho-proteomic analysis, which generated a raw list of about 1000 proteins. Future work will involve the use of an in vivosubstrate trapping method to identify direct PTP1B substrates.From the present study, we hope to determine the molecular mechanisms by which PTP1B regulates ErbB2-induced breast carcinogenesis, with the ultimate aim of identifying new therapeutic targets and new biomarkers for breast cancer.

**15. SUBJECT TERMS**
Breast cancer, ErbB2, MCF10A, Oncogenes, PTP1B, Quantitative Proteomics, Signal Transduction, SILAC

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## INTRODUCTION

The receptor tyrosine kinase ErbB2 is overexpressed in approximately 25% of all breast cancers. ErbB2, and the signaling pathways it activates, represent potential selective targets for therapy in breast cancer. However, drug resistance is a significant clinical problem with current ErbB2-targeted breast cancer therapies. This is because of our lack of understanding of the molecular mechanism underlying the role of ErbB2 in breast carcinogenesis. Recently, Dr. Chernoff and others have reported that a cytoplasmic enzyme, protein tyrosine phosphatase 1B (PTP1B) plays a positive role in ErbB2-induced breast cancer *in vitro* and *in vivo*. Therefore, a detailed understanding of the signaling crosstalk between ErbB2 and PTP1B regulated pathways is warranted. In this research proposal, I have aimed to identify key pro-oncogenic PTP1B regulated pathways in ErbB2 signaling in MCF10A breast epithelial cells using quantitative phospho-proteomics. The identified signaling pathways will be validated in a three-dimensional cell culture system, followed by evaluation in additional ErbB2-transformed breast epithelial cell lines. This study has the potential to uncover novel molecular targets in ErbB2-positive breast cancer and is expected to provide new hope and direction to the present breast cancer biomarkers and therapeutics.

## BODY

In the approved Statement of Work, my first goal was to identify global PTP1B regulated pathways in ErbB2 signaling in MCF10A breast epithelial cells using SILAC (Stable Isotope Labeling by Amino Acids in Cell Culture). SILAC, developed by Mann and colleagues, is an *in vivo* labeling approach where the proteome is labeled as the cells grow in culture [1] and can be used to compare relative changes in protein phosphorylation levels between samples [2]. This task had been projected to be completed within a timeframe of approximately 18 months. This has been accomplished and the following actions as outlined in the Statement of Work were executed successfully:

i) PTP1B was knocked down in 10A.ErbB1:B2 cells [3] (MCF10A cells expressing a chimeric, rapalog-stimulatable form of ErbB1:ErbB2) by stable transfection with a PTP1B specific siRNA expression construct [4]. Stable transfection of 10A.ErbB1:B2 cells with a scrambled siRNA construct was used as the control. Knockdown of PTP1B was confirmed to be >75% in the appropriate cell line by assessing PTP1B levels by Western Blots (Figure 1).

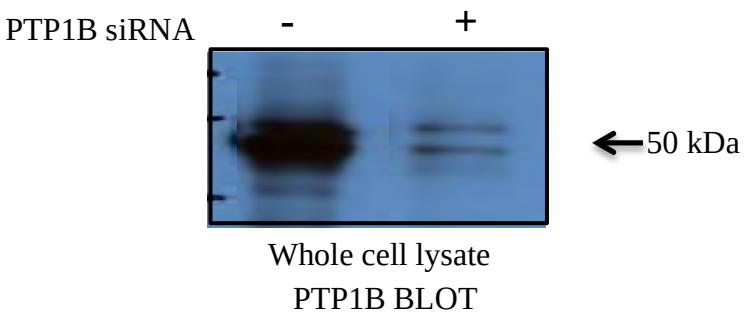


**Figure 1:** Western Blot analysis of 10A.ErbB1:B2 whole cell lysates with PTP1B monoclonal antibody.

ii) The cell population with normal PTP1B expression (ErbB1:B2/PTP1B+) was labeled with media containing heavy isotopes of Lys and Arg (C13 labeled Lys and C13 labeled Arg). The cell population with reduced PTP1B expression (ErbB1:B2/PTP1B-) was labeled with media containing light or normal isotopes of Lys and Arg. After labeling of the two cell populations with appropriate SILAC media, the chimeric ErbB1:ErbB2 in the two populations was activated with the hetero-dimerizing agent, rapalog.

iii) The lysates from the differentially labeled cells (ErbB1:B2/PTP1B+ and ErbB1:B2/PTP1B-) were pooled and the phosphotyrosyl proteins were immunoprecipitated with agarose-conjugated antiphosphotyrosine 4G10 Platinum monoclonal antibody from Millipore. The phosphotyrosyl proteins were eluted using phenyl phosphate, followed by SDS-PAGE analysis.

iv) Protein bands were cut out for trypsin digestion; and the phosphotyrosyl tryptic peptides analyzed using LC-MS/MS with the help of Dr. Steven Seeholzer, Director of the Protein Core Facility at the Childeren's Hospital of Philadelphia (CHOP), who has expertise with various proteomic techniques. Analysis of mass spectra results was done using MASCOT and peptide quantification using MSQUANT open source software [5].

Earlier studies on ErbB2 signaling in NIH3T3 fibroblasts using similar approach identified about 200 proteins including previously known as well as unknown proteins [6]. A similar number were identified in SILAC study using PTP1B wild type and knock out mouse embryonic fibroblasts [7]. The present study generated a raw list of about 1000 proteins. Future work will involve the use of an *in vivo* substrate trapping method to distinguish between direct and indirect PTP1B substrates, followed by validation of the identified PTP1B substrates in bringing about the proliferative and morphogenetic effects of ErbB2 signaling in breast cancer.

**KEY RESEARCH ACCOMPLISHMENTS**

- A stable knockdown of PTP1B was generated in 10A.ErbB1:B2 cells and confirmed by Western Blot analysis.

- SILAC study was performed for the very first time in mammary epithelial cells, which revealed about 1000 proteins which could potentially be involved in PTP1B regulated pathways. However, this requires further validation.

**REPORTABLE OUTCOMES**

- A stable knockdown of PTP1B was generated in 10A.ErbB1:B2 cells
- The work done so far was presented as a poster at the 2011 Era of Hope Conference on Breast Cancer held in Orlando from Aug 2-5, 2011
- The present work resulted in a database of proteins which could potentially be regulated by PTP1B in response to ErbB2 signaling in mammary epithelial cells

**CONCLUSIONS**

This research project aims to enhance our knowledge database about the role of PTP1B in oncogenic signal transduction, with specific focus on breast cancer. Importantly, I intend to validate the contribution of PTP1B to ErbB2-induced breast cancer using human mammary epithelial cells. An understanding of the detailed molecular mechanism by which PTP1B regulates ErbB2-induced breast carcinogenesis is expected to provide new hope and direction to the present breast cancer therapeutics. An in depth understanding of the molecular pathways leading to the development of breast cancer (as is expected from the present study) can help in the identification of new biomarkers for breast cancer, in addition to identifying new targets for breast cancer therapeutics.

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**APPENDICES**

None at this point.